

## REVIEW ARTICLE

## Hereditary paraganglioma targets diverse paraganglia

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Paragangliomas are highly vascularised and often heritable tumours derived from paraganglia, a diffuse neuroendocrine system dispersed from skull base to the pelvic floor. The carotid body, a small oxygen sensing organ located at the bifurcation of the carotid artery in the head and neck and the adrenal medulla in the abdomen, are the most common tumour sites. It now appears that mutations in *SDHB*, *SDHC*, and *SDHD*, which encode subunits of mitochondrial complex II (succinate dehydrogenase; succinate-ubiquinone oxidoreductase), are responsible for the majority of familial paragangliomas and also for a significant fraction of non-familial tumours. Germline mutations in complex II genes are associated with the development of paragangliomas in diverse anatomical locations, including pheochromocytomas, a finding that has important implications for the clinical management of patients and genetic counselling of families. Consequently, patients with a paraganglioma tumour, including pheochromocytoma, and a complex II germline mutation should be diagnosed with hereditary paraganglioma, regardless of family history, anatomical location, or multiplicity of tumours. This short review attempts to bring together relevant genetic data on paragangliomas with a particular emphasis on head and neck paragangliomas and pheochromocytomas.

Paragangliomas are rare and unusual tumours. The ongoing interest in paragangliomas is largely driven by atypical facets of their biology, including neurogenic origin, potential to develop throughout the body, endocrine activity, induction by chronic hypoxic exposure, a high proportion of heritable cases, imprinted familial transmission pattern, and inherited mitochondrial complex II defects in the aetiology. Paragangliomas arise from paraganglia, a collection of neuroendocrine tissues and small organs with a common embryological origin and histological structure that are distributed throughout the body, starting from the middle ear and the skull base and extending into the pelvic floor.<sup>1</sup> The normal paraganglia play important roles in organismic homeostasis either by acting directly as chemical sensors or by secreting catecholamines in response to stress.

The systemic distribution of paragangliomas leads to their recognition and management by multiple medical disciplines. Paragangliomas have been traditionally evaluated in two categories:

those in the head and neck region with the carotid body as the major site and those located below the head and neck with the adrenal medulla as the major site. This anatomical distinction also reflects differences in autonomic functions and the endocrine activity of normal paraganglia. The paraganglia in the head and neck region are anatomically associated with the parasympathetic nervous system and are located in the vicinity of major arteries and nerves, whereas the adrenal medulla and other paraganglia below the head and neck are more closely associated with the sympathetic nervous system. The carotid body (CB) and other head and neck paraganglia often lack the endocrine activity possessed by the adrenal medulla. Consequently, while most patients with head and neck paragangliomas (HNPs) present with an asymptomatic, slow growing mass, patients with pheochromocytomas that often originate from the adrenal medulla are recognised by hypertensive crises and paroxysmal symptoms induced by high circulating catecholamines. The endocrine activity forms the basis of chromaffin reaction displayed by the catecholamine secreting cells of adrenal medulla, in the presence of dichromate containing fixatives. On the basis of negative or equivocal chromaffin reaction of the CB cells, the head and neck paraganglia are sometimes referred to as non-chromaffin paraganglia, although lesser amounts of catecholamines are also present in the CB.

Paraganglia throughout the body have similar morphology and, as discussed below, they have some functional overlap. The unity of the paraganglionic system has been further suggested by the observation that germline mutations in mitochondrial complex II subunits predispose to both HNPs and pheochromocytomas. These findings suggest that paragangliomas in diverse locations can be induced by common pathophysiological mechanisms triggered by complex II gene defects. This brief review will attempt to converge data on the genetic aspects of paragangliomas with particular emphasis on the HNPs and pheochromocytomas, the two most common tumour locations.

## AN OVERVIEW OF PARAGANGLIOMAS

## Head and neck

Zak and Lawson<sup>2</sup> listed nearly 20 distinct anatomical locations for the head and neck paraganglia. The carotid body is the major paraganglion and is also the most common tumour location. Other common locations include jugular, vagal,

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**Abbreviations:** PGL, hereditary paraganglioma; CB, carotid body; HNP, head and neck paraganglioma; VHL, von Hippel-Lindau disease; MEN2, multiple endocrine neoplasia type 2; NF1, neurofibromatosis 1; LOH, loss of heterozygosity

tympenic, and aortic paraganglia. HNPs derive from the hormonally silent non-chromaffin paraganglia, which primarily have sensory innervation and function as chemoreceptors. Almost every physiological and pathological parameter of the CB has been linked to its oxygen sensing function.

The CB senses hypoxia and subsequently induces an increase in the ventilation and heart rates by stimulating the central respiratory centres via afferent glossopharyngeal nerves. Although a large body of data is available on the molecular mechanisms of oxygen sensing and signalling in the CB, how reduced oxygen levels are initially sensed and which molecule(s) is involved is unknown.<sup>3</sup> The neoplastic enlargement of the CB has been linked to chronic hypoxic stimulation of the oxygen sensing chief cells.<sup>4</sup> This observation led to the hypothesis that the mitochondrial complex II, defective in PGL, may be pivotal for CB's oxygen sensing. The readers are referred to Baysal<sup>5</sup> for a more detailed discussion of the response of the CB to chronic hypoxia vis à vis PGL and other genetic epidemiological aspects of HNPs.

### Adrenal medulla

The paragangliomas located below the head and neck most commonly occur in the adrenal medulla and are conventionally referred to as pheochromocytomas. The physiological function of the adrenal medulla is to help the organism to cope with stresses such as cold, hypoglycaemia, haemorrhage, immobilisation, and hypoxia, by secreting catecholamines (dopamine, adrenaline, and noradrenaline).<sup>6</sup> Thus, the adrenal medulla contributes to the organism's "fight or flight" response mediated by the sympathetic nervous system. Extra-adrenal paraganglionic cell clusters and organs include the organ of Zuckerkandl, prevertebral and paravertebral thoraco-abdominal and pelvic paraganglia, and other ganglia in ovary, testis, vagina, urethra, prostate, bladder, and liver.<sup>2</sup>

Most catecholamine secretion from adult adrenal medulla occurs following nervous stimulation by preganglionic fibres or in response to circulating hormones. However, both normal<sup>7</sup> and neoplastic<sup>8</sup> adrenal medullary cells possess direct hypoxia responsiveness. For example, in sheep fetus<sup>9</sup> and rat newborn,<sup>10</sup> hypoxia acts directly on the adrenal medullary cells to stimulate catecholamine secretion before the development of a functional innervation. It is also suggested that extra-adrenal paraganglia, such as the organ of Zuckerkandl, which lacks extensive innervation and functions as the major source of catecholamines in utero, may also be stimulated directly by hypoxia.<sup>11</sup> Thus, although paraganglia in the adult differ in their anatomical location, endocrine activity, and innervation, they are united by their common embryological origin, similar morphology, hypoxia responsiveness, and a role in acute organismic homeostasis.

### INCIDENCE OF PARAGANGLIOMAS

Both head and neck and abdominal paragangliomas are extremely rare. A limited amount of population data is available to obtain a reliable estimate of paraganglioma incidence per year, especially for those located in the head and neck. Further complicating the assessment of the true incidence is the increased frequency of HNPs among high altitude dwellers. The incidence of HNPs appears to be 10 times higher among residents of the Andean mountains.<sup>12</sup> It is also almost certain that the clinical incidence of paragangliomas is lower than the necropsy incidence owing to the often asymptomatic and clinically favourable nature of the tumours. A population based surgical incidence of 1/1 000 000 was reported for HNPs by Oosterwijk *et al*<sup>13</sup> in reference to total surgically treated cases in The Netherlands. Higher necropsy incidences of 1/3860<sup>14</sup> and 1/13 400<sup>15</sup> were reported for carotid body paragangliomas. Comparison of surgical and necropsy incidences suggests that most HNPs are not clinically recognised or operated upon.

More studies have been conducted on the population based clinical incidence of pheochromocytomas per year. The estimates vary between 0.4<sup>16</sup> to 9.5<sup>17</sup>/1 000 000. Other reported estimates are 1.55,<sup>18</sup> 1.9,<sup>19</sup> 2.06,<sup>20</sup> and 2.1<sup>21</sup>/1 000 000 with a median value of ~ 2/1 000 000. The necropsy incidences are higher within a range of 1/742<sup>22</sup> to ~1/2050.<sup>23 24</sup> Thus, both clinical and necropsy incidences of pheochromocytomas appear to be several fold higher than those of HNPs.

The clinical incidence of paragangliomas other than the HNPs and pheochromocytomas is less clear. An estimate of such non-head and neck, non-pheochromocytoma paragangliomas could be obtained from the distribution of tumours in 236 paraganglioma patients.<sup>25</sup> This study suggests that the frequency of such atypically located paragangliomas is nearly 45% of that of HNPs. Thus, the yearly clinical incidence of all paragangliomas (HNPs + pheochromocytomas + other paragangliomas) can be approximately estimated as (1+2+0.45)/1 000 000 which corresponds to ~ 1/300 000. This is likely to be a conservative estimate and the yearly incidence may be expected to rise with the advent of better screening methods and clinical awareness.

### PROPORTION OF FAMILIAL PARAGANGLIOMAS

The proportion of HNPs with a positive family history has been variably estimated from 9.5% in a US clinical sample<sup>26</sup> to 50% in a Dutch clinical sample.<sup>27</sup> A recent analysis of an unselected set of patients managed in two US clinics uncovered a positive family history in ~25% of HNPs.<sup>28</sup> An additional 10% of patients reported multiple head and neck tumours without a family history. In most familial cases of HNPs, there is no report of increased frequency of non-paraganglionic tumours. Carney's triad, the association of paragangliomas with gastric leiomyosarcoma and pulmonary chondroma, may have a distinct aetiology and there is limited evidence to suggest a familial basis for this rare condition.<sup>29</sup> Recently, a new autosomal dominant syndrome characterised by paragangliomas and gastric stromal sarcomas and distinct from Carney's triad has also been described.<sup>30</sup> HNPs were occasionally observed in von Hippel-Lindau disease (VHL),<sup>31-34</sup> multiple endocrine neoplasia type 2 (MEN2),<sup>35 36</sup> and neurofibromatosis 1 (NF1).<sup>37</sup> However, no "HNP only" family has so far been reported with a germline mutation in *VHL*, *RET*, or *NFI*, the causative genes of these multitumour syndromes.

Pheochromocytomas are frequently observed in kindreds with VHL, MEN2, or, less frequently, NF1.<sup>38 39</sup> Familial cases of pheochromocytomas without any syndromic stigmata have also been described.<sup>40 41</sup> It has been suggested that up to 50% of such isolated familial pheochromocytomas<sup>42 43</sup> can be caused by certain missense mutations in the *VHL* gene.

### THE GENETIC BASIS OF FAMILIAL PARAGANGLIOMAS

Germline mutations in the mitochondrial complex II genes, *SDHB*, *SDHC*, and *SDHD*, cause hereditary paraganglioma (PGL) (table 1). *SDHB* (*PGL4*) at chromosome 1p36,<sup>44</sup> *SDHC* (*PGL3*) at chromosome 1q21,<sup>45</sup> and *SDHD* (*PGL1*) at chromosome 11q23<sup>46</sup> encode three subunits of mitochondrial complex II (succinate dehydrogenase; succinate-ubiquinone oxidoreductase), a heterotetrameric complex that is involved in the aerobic electron transport chain and in the Krebs cycle.<sup>47</sup> *SDHD* was the first gene to be mapped<sup>48</sup> and positionally cloned in PGL families.<sup>46</sup> The *SDHD* gene is composed of four exons and encodes a protein of 159 amino acid. *SDHC* was independently mapped<sup>49</sup> and a mutation was identified in a single German PGL family.<sup>45</sup> The *SDHC* gene is composed of six exons and encodes a protein of 169 amino acids. *SDHB* mutations were identified by candidate gene analysis and direct sequencing in small families without previous gene mapping.<sup>44</sup> The *SDHB* gene is composed of eight exons and encodes a protein of 280 amino acids. Another PGL locus, mapped to chromosome

**Table 1** Mutations in PGL genes

Gene	cDNA mutations	Protein change	Phenotype	Reference
<i>SDHB (PGL4)</i>	c.79 C>T	R27X	N-Phaeo	77
	c.86-87 insCAG	A29-Q30 insQ	N-Phaeo	77
	*c.136 C>G	R46G	N-Phaeo	77
	c.174-175 GC>TT	Q59X	N-HNP	62
	c.207-210 insC	M71fs	F-HNP	62
	*c.268 C>T	R90X	F-Phaeo+HNP	44
	*c.302 G>A	C101Y	N-Phaeo	77
	c.392 C>G	P131R	F-HNP	62
	c.574 T>C	C192R	N-Phaeo	77
	c.587 G>A	C196Y	N-Phaeo	77
	c.590 C>G	P197R	F-Phaeo	44
	c.591 delC	P197fs	N-Phaeo	44
	*c.713-718 delTCTC	L240fs	N-Phaeo	77
	c.725 G>A	R242H	N-Phaeo	77
	c.747 C>A	C249X	N-Phaeo	77
<i>SDHC (PGL3)</i>	c.3 G>A	?Aberrant initiation	F-HNP	45
<i>SDHD (PGL1)</i>	c.1 G>C	?Aberrant initiation	F-HNP	59
	IVS1+2 T>G	?Aberrant splicing	N-Phaeo	77, 79
	c.36-37 delTG	A13fs	N-Phaeo	77
	c.14 G>A	W5X	N-Phaeo	77
	*c.33 C>A	C11X	N-Phaeo	77
	c.54 insC	A18fs	F-HNP	60
	*c.64 C>T	R22X	F-HNP+phaeo	55, 60
	(c.95 C>T)	S32X	F-HNP	58
	c.94-97 delCT	S32fs	F-Phaeo	61
	c.106 C>T	Q36X	F-HNPs	46
	*c.112 C>T	R38X	F-HNP, N-phaeo	46, 60, 62, 77, 79
	c.120 insC	P41fs	F-HNP	60
	c.191-192 delTC	L64fs	F-HNP	59
	c.208 A>G	R70G	F-HNP	60
	*c.242 C>T	P81L	F-HNP, N-HNP	46, 58–60, 62
	*c.274 G>T	D92Y	F-HNP, N-HNP, N-Phaeo	46, 60, 77
	c.276-278 delCTA	Del Y93	F-HNP	59
	c.284 T>C	L95P	N-HNP	60
	c.305 A>T	H102L	F-HNP	46
	c.325 C>T	Q109X	F-HNP	62
	(c.336-337 insT)	D113X	F-HNP	58
	c.361 C>T	Q121X	N-Phaeo	77
	(c.341 A>G)	Y114C	F-HNP	58
	c.381-383 delG	L128fs	F-HNP	62
	*c.416 T>C	L139P	N-HNP, F-HNP	60
	(c.441-443 delG)	G148fs	F-HNP	58
	c.149 A>G	H50R, polymorphism?	MC, MCC	67

The cDNA numbering starts with the first nucleotide of the initiation codon.

The nucleotide changes in parentheses were deduced from the protein changes.

\*Mutations described in multiple unrelated subjects.

F-, familial; N-, non-familial; HNP, head and neck paraganglioma; Phaeo, pheochromocytoma; MC, midgut carcinoid; MCC, Merkel cell carcinoma.

11q13 (*PGL2*) by linkage in an extended Dutch family,<sup>50</sup> remains unconfirmed. No other loci have been implicated in familial paragangliomas. Germline mutations in *SDHA*, the fourth subunit of mitochondrial complex II, results in an entirely different phenotype characterised by optic atrophy, ataxia, myopathy, and Leigh syndrome.<sup>51–53</sup> Possible mechanisms of this phenotypic dichotomy resulting from complex II mutations have been recently discussed.<sup>54</sup>

The nature of the germline mutations in complex II subunits (table 1) predicts loss of function of the mutant variants.<sup>54</sup> The subsequent somatic loss of the non-mutant alleles (that is loss of heterozygosity, LOH) in the tumours<sup>45–46</sup> suggests that these genes function as tumour suppressors in the paraganglionic system. However, the molecular steps linking loss of complex II subunits to cellular proliferation are unknown. It has recently been shown that complex II activity is selectively and completely lost in PGL tumours with *SDHD* mutations.<sup>55</sup> The subsequent overexpression of several hypoxia inducible genes in paragangliomas<sup>55–56</sup> is in harmony with the hypothesis that loss of complex II mimics chronic hypoxic stimulation, which leads to adaptive proliferation of the paraganglia.<sup>5</sup>

The transmission pattern of *SDHD* mutations shows strict parent of origin effects.<sup>27–48–57</sup> The disease phenotype is transmitted only through the fathers, whereas mothers do not

transmit the disease. This transmission pattern has not yet been violated in any PGL family with an *SDHD* mutation.<sup>55–58–62</sup>

The presence of *SDHD* founder mutations<sup>53–64</sup> clearly indicates that this sex specific transmission effect operates over multiple generations and therefore is reversible in gametes. This observation strongly suggests that *SDHD* is subject to genomic imprinting, although the exact molecular mechanisms remain unknown. The *SDHD* gene shows biallelic expression in various tested tissues.<sup>46</sup> There is no evidence for a parent specific disease transmission in families with *SDHC* and *SDHB* mutations.<sup>44–62–65</sup> This suggests that the sex specific transmission pattern in PGL1 is not the result of an obscure functional mechanism induced by loss of complex II, but a locus specific epigenetic phenomenon operating on the *SDHD* gene.

## PREVALENCE OF MITOCHONDRIAL COMPLEX II GERMLINE MUTATIONS IN PARAGANGLIOMAS

### Head and neck paragangliomas

#### Familial head and neck paragangliomas

In the presence of a positive family history the germline mutations in *SDHD*, *SDHB*, and *SDHC* account for the majority of the HNPs. *SDHD* is the most commonly mutated gene with 26 distinct mutations described so far (table 1). Almost all

HNPs with a positive family history are caused by two *SDHD* founder mutations (D92Y and L139P) in The Netherlands.<sup>60</sup> The genomic imprinting effect at the *SDHD* locus may have helped the spread of the founder mutations by effectively halving the overall penetrance of the mutant alleles. In contrast, only 50% of the familial HNPs harboured *SDHD* germline mutations in the US based sample.<sup>62</sup> There is a founder effect among the US patients who carry the *SDHD* P81L mutation.<sup>46–64</sup> However, because P81L and R38X are also potentially recurrent mutations,<sup>62</sup> some subjects with the P81L mutation may not carry the founder mutation. The founder effect and the recurrent mutational mechanisms make P81L the first mutation to be tested in screening familial HNPs in North America.

Another 20% of the familial HNPs are caused by *SDHB* germline mutations in the US sample.<sup>62</sup> Only a few *SDHB* linked HNP families have been described so far.<sup>44–62</sup> These families, unlike the *SDHD* linked ones, are small with only two or three affected subjects. Although recurrent mutations, such as R90X, were reported in unrelated families, there is no evidence for a founder mutation in *SDHB*.<sup>44</sup> These findings suggest that *SDHB* mutations may be associated with poorer phenotypic fitness. This may lead to a quick removal of the mutant alleles from the population gene pool and to the observation of families with few affected subjects owing to early recognition of the mutation carriers. Complex II mutations could not be identified in up to 30% of familial HNPs in the US sample.<sup>62</sup> This could be partly because of the PCR based approaches used for mutation screening, which could miss certain gene defects such as large deletions and rearrangements. It is also noteworthy that no additional mutations have been found in the *SDHC* gene since the discovery of an initiation codon mutation in a large German family.<sup>45</sup> Whether unconventional mutational mechanisms are operative particularly on *SDHC* is unknown. There is no confirmed evidence to suggest involvement of non-complex II genes in the aetiology of familial HNPs.

### Non-familial head and neck paragangliomas

In the absence of positive family history, complex II mutations still play significant roles in the aetiology of HNPs. In The Netherlands, ~36% of the non-familial cases carry the two founder *SDHD* mutations.<sup>60</sup> Germline mutations in complex II genes were less frequent in the non-familial HNPs in the US sample: *SDHD* and *SDHB* mutations were found in 5% and 3% of the subjects, respectively.<sup>62</sup> Although these percentages may increase with the use of more comprehensive mutation screening methods, it is possible that some, perhaps most, non-familial HNPs may have distinct and non-genetic aetiologies.

### Phaeochromocytomas

#### Familial phaeochromocytomas

Identification of complex II germline mutations in familial phaeochromocytomas suggests that PGL may manifest itself predominantly with non-HNPs in certain pedigrees. Phaeochromocytoma was the only finding in four subjects in a family with *SDHD* mutation<sup>61</sup> and in four subjects from two families with *SDHB* mutations,<sup>44</sup> suggesting involvement of complex II genes in the aetiology of rare non-syndromic familial phaeochromocytomas. However, two recent studies failed to identify *SDHD* mutations in 10 familial phaeochromocytomas.<sup>66–67</sup> Interestingly, in three families with *SDHD* mutations, the phaeochromocytomas were not confined to the adrenal gland, but other abdominal paraganglia as well as HNPs in some subjects were involved.<sup>44–61</sup> The presence of subjects with HNPs in some of the *SDHD* and *SDHB* mutant familial phaeochromocytoma pedigrees indicates that identical mutations can induce tumours both in the head and neck and in the abdominal region. Whether anatomical location of paragangliomas is influenced by a specific complex II

mutation remains to be established. Phaeochromocytomas were reported in only three of 63 (~4.8%) Dutch familial HNP subjects,<sup>65</sup> suggesting that mutations in *SDHD*, at least for the two Dutch founder mutations, are infrequently associated with abdominal involvement.

### Non-familial phaeochromocytomas

Phaeochromocytomas presenting without a family history of VHL, MEN2, or NF1 can be associated with germline mutations in the *VHL* and *RET* genes. The estimates of prevalence of occult germline mutations in *RET* in non-familial phaeochromocytomas include 0/10 (0%),<sup>68</sup> 1/120 (~0.8%),<sup>69</sup> 6/28 (~21%),<sup>70</sup> 1/48 (~2.1%),<sup>71</sup> 0/29 (0%),<sup>72</sup> 0/27 (0%),<sup>73</sup> 1/12 (~8.3%),<sup>72</sup> 0/62 (0%),<sup>74</sup> 0/5 (0%),<sup>75</sup> 0/7 (0%),<sup>76</sup> and 13/271 (~4.8%).<sup>77</sup> The prevalence estimates of occult germline mutations in *VHL* in non-familial phaeochromocytomas are somewhat higher, including 1/48 (~2.1%),<sup>71</sup> 1/5 (20%),<sup>75</sup> 2/62 (~3.2%),<sup>74</sup> 1/27 (~3.7%),<sup>73</sup> 6/68 (~8.8%),<sup>78</sup> 30/271 (~11%).<sup>77</sup> Although these studies are not identical in their research methodologies, when all data are combined the prevalences of occult germline mutations in *RET* and *VHL* in non-familial phaeochromocytomas are estimated as 22/619 (~3.6%) and 41/481 (~8.5%), respectively.

Analysis of non-familial phaeochromocytomas by Gimm *et al*.<sup>79</sup> uncovered *SDHD* germline mutations in two of 18 (~11%) subjects. One subject with *SDHD* mutation later developed head and neck tumours. Recently, analysis of *SDHD* in 271 non-familial phaeochromocytomas showed germline mutations in 11 cases. Three other studies, however, failed to identify any germline *SDHD* mutations in a total of 80 non-familial phaeochromocytomas.<sup>61–66–67</sup> These studies suggest a combined *SDHD* mutation rate of 13/369 (~3.5%) in non-familial phaeochromocytomas.

Mutations of the *SDHB* gene were uncovered in 12/271 (~4.4%)<sup>77</sup> and 1/20 (5%)<sup>44</sup> non-familial phaeochromocytomas with a combined estimate of 13/291 (~4.5%). Altogether these preliminary findings suggest that ~8.0% of non-familial phaeochromocytomas may harbour occult germline mutations in *SDHD* and *SDHB*. These findings also suggest that co-occurrence of head/neck or extra-adrenal paragangliomas, either in the same person or in other family members, may be indicative of germline mutations in mitochondrial complex II genes.

### Other paragangliomas and tumour types

The mitochondrial complex II genes are obvious candidates for paragangliomas observed in atypical locations and in other more common tumour types that show LOH at 11q23 and 1p36. For example, spinal paragangliomas are exceedingly rare tumours of unknown origin and pathogenesis.<sup>80</sup> They most commonly occur at the level of the cauda equina and do not appear to be associated with other paragangliomas. Furthermore, the spinal cord is not one of the known tumour locations in PGL.<sup>63</sup> Analysis of the *SDHD* gene in 20 spinal paragangliomas showed G12S variant in one subject with a cauda equina tumour.<sup>81</sup> G12S and H50R variants were also detected among midgut carcinoids and Merkel cell carcinomas.<sup>67</sup> These missense variants, however, do not meet stringent criteria to qualify as a mutation: they are located in the mitochondrial signal peptide, are not conserved across species, and are not known to cosegregate with the disease phenotype in any family. Furthermore, G12S, which was first identified in the germline of a non-familial phaeochromocytoma patient,<sup>79</sup> has been observed both in heterozygous and homozygous conditions in phenotypically normal subjects.<sup>60</sup> Thus, further studies are needed to prove that G12S and H50R have phenotypic consequences. Finally, analysis of the *SDHD* gene in 43 primary nasopharyngeal carcinomas, which show a high degree of LOH at 11q23, did not show any germline mutations, but a novel P81P silent variant.<sup>82</sup>

## THE CLINICAL INCIDENCE OF PGL

The clinical incidence of all paragangliomas and the preliminary data on the prevalence of *SDHD* and *SDHB* germline mutations of the mitochondrial complex II genes now allow us to estimate the clinical incidence of PGL. The incidence of paragangliomas excluding pheochromocytomas caused by complex II germline mutations is clinical incidence  $(1.45/1\,000\,000) \times [\text{proportion of familial paragangliomas } (0.25) \times \text{frequency of germline mutations } (0.7) + \text{proportion of non-familial paragangliomas } (0.75) \times \text{frequency of germline mutations } (0.08)]$ , which equals  $\sim 0.34/1\,000\,000$ . The incidence of pheochromocytomas caused by complex II germline mutations is clinical incidence  $(2/1\,000\,000) \times \text{frequency of germline mutation } (0.08)$ , which equals  $0.16/1\,000\,000$ . Thus, the total yearly clinical incidence of all paragangliomas caused by *SDHD* and *SDHB* mutations, that is, PGL, is estimated as  $\sim 1/2\,000\,000$ .

## SUMMARY AND CONCLUSIONS

The development of paragangliomas in diverse anatomical locations in subjects with *SDHB*, *SDHC*, and *SDHD* germline mutations indicate that the paraganglionic system throughout the body is a target in PGL. Thus, the possibility of complex II germline mutations should be raised in the differential diagnosis of all paragangliomas including pheochromocytomas. Whether certain subunit mutations are more strongly associated with a given anatomical location, hormonal activity, malignancy, age at onset, tumour multiplicity, and size remains to be established. Other genetic loci and environmental factors may also affect phenotypic expression. So far, there is no evidence for increased tumour susceptibility in non-paraganglionic tissues in PGL.

Germline mutations in *SDHB*, *SDHC*, and *SDHD* account for the majority of paragangliomas if there is a positive familial history and *SDHD* appears to be the most commonly mutated gene. Approximately 8% of all non-familial paragangliomas also harbour occult germline mutations in the complex II genes. Multiple paragangliomas in non-familial cases, regardless of their anatomical distribution, and, because of the rarity of paragangliomas, the presence of two or more first or second degree affected relatives should herald the possibility of complex II mutations. However, more studies are required to determine whether subjects with solitary paragangliomas and no family history are likely to have complex II germline mutations. In a recent study, 45 of 66 (68%) non-familial pheochromocytoma patients who had germline mutations in *VHL*, *RET*, *SDHD*, or *SDHB* presented with solitary tumours.<sup>77</sup> The high combined rate of germline mutations in these genes in non-familial pheochromocytomas may therefore justify gene testing in subjects presenting with a single pheochromocytoma tumour. However, complex II gene mutations were discovered only in one of 32 ( $\sim 3\%$ ) subjects with non-familial HNPs presenting with solitary tumours.<sup>62</sup> Thus, a recommendation for gene testing seems to be currently premature for sporadic patients presenting with a single HNP.

Genetic analysis has the potential to offer new opportunities in the clinical management of the paraganglioma patients and their families. If germline mutations in complex II genes are uncovered in a patient, a clinical search for additional paragangliomas may be warranted. Early detection and removal of a critically located HNP or a hormonally active pheochromocytoma may help reduce the risk of morbidity and mortality.

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